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Prevalence and Risk Factor Analysis of TTV Infection in Prostitutes

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TTV, a DNA virus, has been isolated from patients with non-A to non-E post-transfusion hepatitis. In the past it was assumed that TTV was transmitted parenterally. It is unclear whether sexual contact leads to transmission of this virus. In this study, two sets of TTV-specific polymerase chain reaction primers were used to detect serum TTV DNA in 140 prostitutes and 136 controls. The prevalence of TTV DNA in prostitutes was significantly higher than in the control group (46/140 [32.9%] vs. 29/136 [21.3%]; $P = 0.043$). There was no significant difference in the prevalence of positive antibody to hepatitis A virus (anti-HAV) in either group (87.8% for prostitutes, 85.3% for controls). No particular risk factor was significantly associated with positive TTV DNA in prostitutes. In summary, TTV is highly prevalent in prostitutes. Transmission of TTV via sexual contact is not as efficient as transmission of hepatits C and D viruses and GB virus-C hepatitis G virus. The high prevalence of TTV in controls indicates that there are diverse routes of transmission of this virus. ***J. Med. Virol. 60:393–395, 2000.***

KEY WORDS: sexual transmission; Parvoviridae; Circoviridae; TT virus; prostitutes

INTRODUCTION

TT virus (TTV) was isolated originally from patients with cryptogenic post-transfusion hepatitis [Nishizawa et al., 1997]. TTV is an unenveloped, single-stranded DNA virus assumed to be related to the parvovirus family [Okamoto et al., 1998a]. More recent studies suggest that the virus resembles the Circoviridae [Mushahwar et al., 1999], viruses that infect plants and vertebrates. In the past, TTV was considered to be transmitted through transfusion as well as other parenteral routes [Naoumov et al., 1998; Okamoto et al., 1998a; Simmonds et al., 1998]. However, the evidence that TTV viremia is highly prevalent in healthy adults and blood donors in the United Kingdom (10%) [Naoumov et al., 1998], Thailand (36%) [Tanaka et al., 1998], Brazil (62%) [Niel et al., 1999], and the general population in developing countries, such as Gambia (83%) [Prescott and Simmonds, 1998], indicates that the transmission route for this virus is varied.

Sexual contact is now the major route for horizontal transmission of hepatitis B virus (HBV) among adults in Taiwan. In addition, sexual contact with prostitutes is the most common mode of hepatitis D virus (HDV) infection in Taiwan [Wu et al., 1990; Hou et al., 1993; Wu et al., 1993a]. Although hepatitis C virus (HCV) is not transmitted as efficiently by sexual contact as HBV or HDV, the prevalence of HCV infection is still high in prostitutes in Taiwan [Wu et al., 1993b]. Prostitutes are also considered to be a high-risk group for GB virus-C (GBV-C) *hepatitis G virus (HGV)* infection, and the prevalence of GBV-C/HGV viremia in prostitutes is significantly higher than that in a control group studied [Wu et al., 1997]. It is unclear whether sexual contact plays a role in the transmission of TTV.

In this study, TTV DNA was detected from serum samples of a large number of prostitutes by using two sets of TTV-specific polymerase chain reaction (PCR) primers. The prostitutes completed questionnaires regarding potential risk factors associated with viral transmission. Univariate and multivariate analyses were carried out to identify the significant factors associated with TTV infection in prostitutes.

MATERIALS AND METHODS

A total of 140 randomly selected female prostitutes in Taiwan were evaluated for serum TTV DNA. The

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cases of these prostitutes have been described previously [Wu et al., 1993a; 1997]. Serum samples from prostitutes were stored at −70°C until used. The prostitutes completed an interviewer-administered questionnaire, with consent as described previously [Wu et al., 1997]. Briefly, the questionnaire included history of transfusion, dental procedures, injection or acupuncture with nondisposable needles and syringes, intravenous drug abuse, tattoos, ear piercing, and frequency of paid sex. Each subject underwent physical examination, including inspection for tattoos or needle-puncture marks. One hundred thirty-six (20 HBV carriers and 116 noncarriers) age-matched, female nonprostitutes who came in for checkups were included as controls. No one in either group showed positive results for antibody to human immunodeficiency virus (HIV).

Serum TTV DNA was detected by nested PCR using hemi-nested primers, as reported by Naoumov et al. [1998], and a second set of fully nested primers, as described previously [Nishizawa et al., 1997]; these are hereinafter referred to as set I and set II, respectively. Samples were defined as viremic if the results were positive with either or both sets of primers [Desai et al., 1999; MacDonald et al., 1999]. Serum GBV-C/HGV RNA was detected by nested PCR after reverse transcription (RT-PCR) [Wu et al., 1998]. The PCR products were analyzed in 3% agarose gel followed by ethidium bromide staining. Strict procedures were followed to avoid false-positive results [Kwok and Higuchi, 1989]. Serum alanine aminotransferase (ALT) was measured by a sequential multi-autoanalyzer (Technicon SMAC; Technicon Instruments, Tarrytown, NY). The following viral markers were assayed by radioimmunoassay kits: IgG antibody to hepatitis A virus (anti-HAV), hepatitis B surface antigen, and anti-HDV (HAVAB, Ausria II-125 and Anti-Delta; Abbott Laboratories, North Chicago, IL). Antibody to hepatitis C virus (anti-HCV) was detected by a second-generation enzyme immunoassay (Abbott Laboratories). Anti-HIV was tested by an enzyme immunoassay kit (Rapid Elavia; Diagnostics Pasteur, Mannes-la-Coquette, France). A Mann-Whitney *U* test and chi-square test with Yates' correction were used for the comparison of various parameters in prostitutes with or without TTV viremia, where appropriate. A P value less than 0.05 was considered significant. Univariate and multivariate analyses were performed using SAS, with logistic regression of factors associated with TTV infection.

RESULTS

TTV DNA was detected in 46 (32.9%) of the 140 prostitutes. Of the 136 control subjects, 29 (21.3%) showed positive results for TTV DNA (three were HBV carriers, and the others were not carriers). The difference in the prevalence rates of TTV viremia between prostitutes and controls was statistically significant (P = 0.043). There was no statistical difference in age between these two groups (prostitutes, 31.9 ± 10.3 years; controls, 34.7 ± 8.7 years). The prevalence rates of positive anti-HAV results in both groups were nearly the same (prostitutes, 123/140 [87.8%]; controls, 116/136 [85.3%]), and the TTV prevalence rates in women with and without anti-HAV were not statistically different (68/239 vs. 7/37). The prevalence of TTV viremia was underestimated when using a single set of primers (Table I).

TABLE I. Comparison of Sensitivity for the Two Sets of TTV PCR Primers

	No. of Positive TTV DNA			
Groups	Set I only	Set II only	Both sets	Total
Prostitutes (n = 140)	21	18	7	46
Controls (n = 136)	12	15	2	29

TTV, TT virus; PCR, polymerase chain reaction.

Univariate and multivariate analyses of risk factors associated with TTV DNA were performed for the prostitutes. As shown in Table II, there were no significant differences in age, serum ALT levels, and prevalence rates of HAV, HBV, HCV, or GBV-C/HGV infection in the prostitutes with or without TTV viremia. Differences of the remaining factors (such as the frequency of paid sex, history of blood transfusion, tattoos, injection with nondisposable needles and syringes, ear piercing, acupuncture, intravenous drug abuse, and dental procedures) were also insignificant.

DISCUSSION

In the past, TTV was considered to be transmitted parenterally [Naoumov et al., 1998; Okamoto et al., 1998a; Simmonds et al., 1998]. There has been no report of sexual transmission of this virus, though our results indicate a significantly higher prevalence rate of TTV in prostitutes. In a recent report that discussed TTV in high-risk groups [MacDonald et al., 1999], the frequency rate of viremia was 13% (7/52) in prostitutes, not significantly different from that in controls (4.5%, 2/44). Davidson et al. [1999] also reported a similar result. A possible reason for this discrepant result is that the numbers of prostitutes and controls in those studies were too small to show any statistical significance. Recent studies using two sets of PCR primers to detect TTV DNA [Desai et al., 1999; MacDonald et al., 1999] show that a single set of primers underestimates the prevalence of TTV viremia. Full-length genomic sequences from variant TTV genotypes are necessary to select a conserved region and to develop consensus primers.

In Taiwan, sexual contact with prostitutes is the most common mode of HDV infection, and the prevalence of HDV in prostitutes is 36- to 55-fold higher than in nonprostitute female carriers (<1%) [Wu et al., 1990, 1993a]. HCV, which is not as efficiently transmitted sexually as HDV, has a sixfold higher prevalence in prostitutes (12%) than in the adult female population (2%) [Wu et al., 1993b]. In a recent study of GBV-C/HGV, the prevalence in prostitutes (21%) was fourfold higher than that in nonprostitute controls (5%) [Wu et

TABLE II. Risk Factor Analysis for TTV Infection in Prostitutes*

Risk factors	TTV DNA positive (n = 46)	TTV DNA negative (n = 94)
Age (mean ± S.D., years)	32.1 ± 11.2	31.8 ± 9.9
ALT (mean ± S.D., U/L)	37.1 ± 28.9	39.8 ± 72.3
No. of cases in persons <20 years old	6 (13.0%)	8 (8.5%)
Positive anti-HAV	42 (91.3%)	81 (86.2%)
Positive HBsAg	13 (28.3%)	15 (16.0%)
Positive anti-HCV	5 (10.9%)	13 (13.8%)
Positive GBV-C/HGV	12 (26.1%)	18 (19.1%)
Monthly paid sex ≥120 times	20 (43.5%)	40 (42.6%)
History of transfusion	1 (2.2%)	6 (6.4%)
Tattoos	21 (45.7%)	41 (43.6%)
Injection using nondisposable needles	8 (17.4%)	8 (8.5%)
History of dental procedures	10 (21.7%)	15 (16.0%)
Ear piercing	28 (60.9%)	59 (62.8%)
History of acupuncture	2 (4.3%)	5 (5.3%)
Intravenous drug abuse	0 (0%)	0 (0%)

*TTV, TT virus; ALT, alanine aminotransferase; U/L, unit/liter; HAV, hepatitis A virus; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; GBV-C, GB virus-C; HGV, hepatitis G virus.

al., 1997]. In the present study, the prevalence of TTV in prostitutes (32.9%) is only one and a half times higher than that among nonprostitute female controls (21.3%). Compared with HCV, HDV, and GBV-C/HGV, TTV seems to have the least transmission efficiency by sexual contact.

Although TTV viremia was highly prevalent in the prostitutes, no particular risk factor was associated with TTV infection. It is noteworthy, however, that the prevalence of TTV in the general population of Taiwan is much higher than that of other parenterally transmitted viruses, such as HCV, HDV, or GBV-C/HGV. Recent studies have found that TTV is prevalent in general populations [Naoumov et al., 1998; Tanaka et al., 1998; Niel et al., 1999], especially in the general populations of developing countries [Prescott and Simmonds, 1998]. These findings are in agreement with the hypothesis that TTV is transmitted predominantly in a nonparenteral pattern. Another important observation, that TTV can be excreted in feces, especially when the host's viral titer is high [Okamoto et al., 1998b], indicates that TTV is probably transmitted by the fecal–oral route. However, the route of transmission for TTV may be distinct from that of HAV, as shown by the lack of correlation between the two viruses. More recent findings are that the molecular and biophysical characteristics of TTV bear a partial resemblance to Circoviridae [Mushahwar et al., 1999], viruses known to infect plants and vertebrates (birds and swine). Farm animals may be a major source of human infection with this virus [Zuckerman, 1999]. TTV viremia does not show a correlation with serum ALT levels. Most of the TTV viremic subjects were asymptomatic for liver disease and had normal or mildly elevated ALT levels. Therefore, the clinical significance of TTV infection is still obscure. In summary, TTV infection is highly prevalent in prostitutes, but it appears to be transmitted relatively inefficiently via sexual contact compared with HCV, HDV, and GBV-C/HGV. The high prevalence rate of TTV in controls indicates that the route of transmission for this virus varies.

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